Effects of selected combinations of tall fescue alkaloids on the vasoconstrictive capacity of fescue-naïve bovine lateral saphenous veins

J. L. Klotz, B. H. Kirch, G. E. Aiken, L. P. Bush and J. R. Strickland

J Anim Sci 2008.86:1021-1028.
doi: 10.2527/jas.2007-0576 originally published online Jan 11, 2008;

Effects of selected combinations of tall fescue alkaloids on the vasoconstrictive capacity of fescue-naïve bovine lateral saphenous veins[1,2]

J. L. Klotz,* B. H. Kirch,* G. E. Aiken,* L. P. Bush,† and J. R. Strickland*[3]

*USDA-ARS, Forage-Animal Production Research Unit, Lexington, KY 40546; and †Department of Plant and Soil Sciences, University of Kentucky, Lexington 40546

ABSTRACT: Vasoconstriction is a response associated with consumption of toxic endophyte-infected tall fescue. It is not known if endophyte-produced alkaloids act alone or collectively in mediating the response. Therefore, the objective of this study was to examine the vasoconstrictive potentials of selected ergot alkaloids, individually or in paired combinations, using bovine lateral saphenous veins biopsied from fescue-naïve cattle. Segments (2 to 3 cm) of vein were surgically biopsied from healthy crossbred yearling heifers (n = 22; 330 ± 8 kg of BW). Veins were trimmed of excess fat and connective tissue, sliced into 2- to 3-mm sections, and suspended in a myograph chamber containing 5 mL of oxygenated Krebs-Henseleit buffer (95% O_2/5% CO_2; pH = 7.4; 37°C). Increasing doses of ergovaline, lysergic acid, and N-acetylloline individually or in combination were evaluated. Contractile data were normalized as a percentage of the contractile response induced by a reference dose of norepinephrine (1×10^{-4} *M*). Increasing concentrations of lysergic acid did not result in an appreciable contractile response until the addition of 1×10^{-4} *M* lysergic acid. In contrast, the vascular response to increasing concentrations of ergovaline was apparent at 1×10^{-8} *M* and increased to a maximum of 104.2 ± 6.0% with the addition of 1×10^{-4} *M* ergovaline. The presence of N-acetylloline did not alter the onset or magnitude of vascular response to either lysergic acid or ergovaline. The presence of 1×10^{-5} *M* lysergic acid with increasing concentrations of N-acetylloline and ergovaline generated an increased contractile response during the initial additions compared with the responses of N-acetylloline and ergovaline alone. In the presence of 1×10^{-7} *M* ergovaline, the contractile response increased with increasing concentrations of N-acetylloline and lysergic acid. Neither N-acetylloline nor lysergic acid elicited an intense contractile response individually (maximum contractile responses of 1.9 ± 0.3% and 22.6 ± 4.1%, respectively), suggesting that this was the result of the repetitive addition of 1×10^{-7} *M* ergovaline. These data indicate that ergovaline is a more potent vascular toxicant than lysergic acid or N-acetylloline. The contractile responses of the ergovaline and lysergic acid combinations appeared to differ from the individual dose responses. These data support the possibility that an additive alkaloid exposure effect may exist and should be considered during evaluations of ergot alkaloids.

Key words: cattle, ergovaline, fescue toxicosis, lysergic acid, N-acetylloline, vasoconstriction

J. Anim. Sci. 2008. 86:1021–1028
doi:10.2527/jas.2007-0576

INTRODUCTION

Ergot alkaloids are chemically diverse and have been shown to result in differing vasoconstrictive responses in herbivores. Specifically, N-acetylloline (unsaturated pyrrolizidine alkaloid; Figure 1A), lysergic acid (ergoline alkaloid; Figure 1B), and ergovaline (ergopeptine alkaloid; Figure 1C) have individually been shown to generate differing vasoconstrictive responses (Abney et al., 1993; Dyer, 1993; Klotz et al., 2006). Because herbivores are exposed to a multiplicity of alkaloids when consuming toxic endophyte-infected tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.; Soreng et al., 2001), a combined alkaloid effect has been suggested

[1]Mention of trade name, proprietary product, or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable.

[2]We wish to thank T. Hamilton and J. Jones of the Forage-Animal Production Research Unit and L. McClanahan, J. Piel, and B. Hightshoe of the University of Kentucky for their assistance in helping to complete the biopsies. Additionally, we would like to acknowledge B. Arrington of the University of Kentucky for his efforts in assisting with the biopsies and his tireless monitoring of the myographs.

[3]Corresponding author: jim.strickland@ars.usda.gov
Received September 11, 2007.
Accepted December 20, 2007.

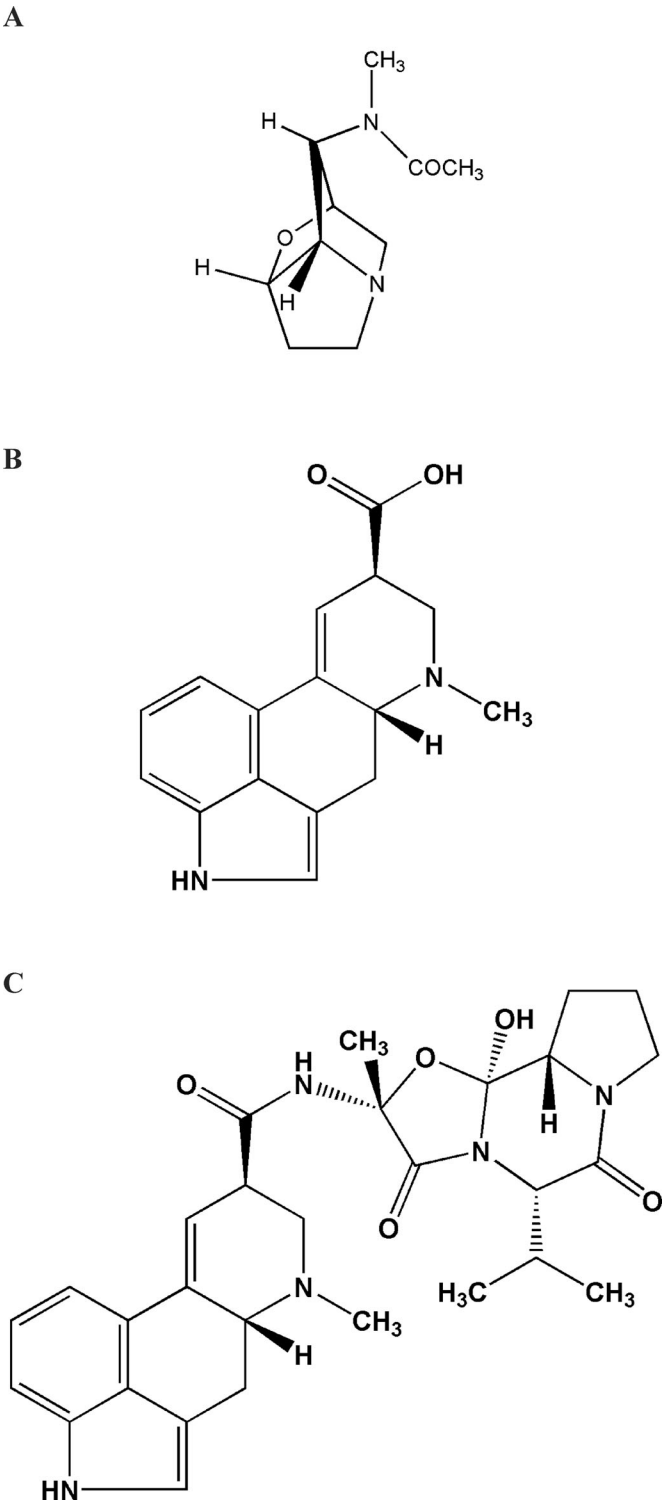

Figure 1. Chemical structures of A) N-acetylloline, B) lysergic acid, and C) ergovaline.

(Oliver, 1997; Moubarak et al., 2003). However, there is presently no documented evidence of vasoconstrictive synergism of tall fescue alkaloids.

In vitro screening of different alkaloids for vasoconstrictive effects has been performed using a bovine lateral saphenous vein bioassay. Previous work using this bioassay has made use of tissue obtained from slaughtered animals (Oliver et al., 1993; Klotz et al. 2006) and from surgical biopsies (Oliver et al., 1998). Although obtaining tissue samples at abattoirs is less complicated, the dietary background of these animals in many cases is unknown. Because it is currently not known if there are long-term or residual vascular effects resulting from previous consumption of toxic endophyte-infected tall fescue, the dietary background of abattoir animals could be a source of variation in research pertaining to fescue toxicosis. Thus, the objective of this experiment was to examine the vasoconstrictive potentials of D-lysergic acid and ergovaline individually and in combination with one another or N-acetylloline using lateral saphenous veins biopsied from fescue-naïve cattle.

MATERIALS AND METHODS

Animals and Tissues

The methods used in this study have been validated and reported by Klotz et al. (2006) and Solomons et al. (1989) and were approved by the University of Kentucky Institutional Animal Care and Use Committee.

Cranial branches of lateral saphenous veins were biopsied from 22 fescue-naïve Angus × Brangus crossbred heifers (obtained from the USDA-ARS, Dale Bumpers Small Farms Research Center, Booneville, AR; 330 ± 8 kg of BW). The heifers were maintained in a drylot on a corn silage diet for the interval leading up to biopsy. Before biopsy, the heifers were placed in a left lateral recumbency using a tilt table (Spring-O-Matic Inc., Marion, KS), and the biopsy site was clipped free of hair, cleaned with povidone-iodine soap solution, disinfected with 70% ethyl alcohol, and locally anesthetized with lidocaine (2% injectible; The Butler Co., Dublin, OH). A 10-cm incision was made through the skin in the tarsal region slightly above and parallel to the cranial branch of the lateral saphenous vein. After s.c. identification of the vessel, ligatures were placed after the division of the lateral saphenous vein into cranial and caudal branches and before the cranial branch merged with a branch of the cranial tibial vein.

The isolated venous tissue was excised and placed in a modified Krebs-Henseleit oxygenated buffer solution (95% O_2/5% CO_2; pH = 7.4; m*M* composition = D-glucose, 11.1; $MgSO_4$, 1.2; KH_2PO_4, 1.2; KCl, 4.7; NaCl, 118.1; $CaCl_2$, 3.4; and $NaHCO_3$, 24.9; Sigma Chemical Co., St. Louis, MO) for transport and kept on ice until processed. Immediately after the biopsy, the heifers received penicillin (Procaine G, 6,600 U/kg of BW; Norbrook Inc., Kansas City, MO) and flunixin meglumine (Flunixiject, 1.1 mg/kg of BW; IVX Animal Health Inc., St. Joseph, MO) and were returned to the drylot for observation. Administration of flunixin meglumine was continued for 2 d postoperatively.

Before conducting biopsies, a preliminary study evaluating the concentration-response to N-acetylloline was conducted separately, using tissue collected from cattle

of mixed breeds and sex (n = 4; BW = 275 to 340 kg) immediately after slaughter at local abattoirs. Other than the dissection procedure, the tissues were obtained, transported, and processed as described for the biopsied vessels. Data from this preliminary study are included with these biopsy data as a justification for not examining N-acetylloline alone with the more difficult to obtain biopsy samples.

Tissue processing consisted of removal of excess fat and connective tissue from the vein segments, which were sliced into 2- to 3-mm cross-sections. Cross-sections were examined under a dissecting microscope (Stemi 2000-C, Carl Zeiss Inc., Oberkochen, Germany) at 12.5× magnification to measure the dimensions for assurance of consistent segment size and to verify the physical integrity of tissue. Duplicate cross-sections from each animal per treatment were suspended horizontally in a 5-mL tissue bath (DMT610M multichamber myograph, Danish Myo Technologies, Atlanta, GA) containing continuously oxygenated modified Krebs-Henseleit buffer (95% O_2/5% CO_2; pH = 7.4; 37°C), with 3×10^{-5} *M* desipramine and 1×10^{-6} *M* propranolol (D3900 and P0844, Sigma Chemical Co.) to inactivate catecholamine-neuronal uptake and β-adrenergic receptors, respectively. After equilibration to 1 g of tension (1.5 h), the tissues were exposed to the α-adrenergic agonist norepinephrine (1×10^{-4} *M*; A0937, Sigma Chemical Co.) to verify tissue viability and as a reference for normalization of the responses.

Alkaloid Concentration-Response Experiments

Cross-sections of the cranial branch of the lateral saphenous vein were run in duplicate from each animal (n = 5 for each alkaloid or combination). After recovery from the 1×10^{-4} *M* norepinephrine addition (45 to 60 min) and the reestablishment of the 1-g baseline tension, the compounds were tested from the least to the greatest concentration in 15-min intervals. Each 15-min interval consisted of a 9-min incubation period followed by a washout period during which the buffer minus the treatment compound was incubated with the tissue for two 2.5-min periods, followed by a final buffer replacement and a 1-min incubation. Increasing concentrations of ergovaline (93%; supplied by Forrest T. Smith, Auburn University, AL), lysergic acid (95%; Acros Organics, Geel, Belgium), and N-acetylloline (USDA, Northern Regional Research Center, Peoria, IL) at 1×10^{-11}, 1×10^{-10}, 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6}, 1×10^{-5}, and 1×10^{-4} *M* were administered individually or in combination. For coincubation experiments, lysergic acid and N-acetylloline were held constant at 1×10^{-5} *M* and ergovaline at 1×10^{-7} *M*, whereas the concentrations of the other alkaloid included in the mixture increased as previously described.

Data Collection and Analysis

Isometric contraction was recorded as grams of tension in response to exposure to norepinephrine and the subsequent alkaloid additions. Data were digitized and recorded using a Powerlab/8sp (ADInstruments, Colorado Springs, CO) and Chart software (Version 5.3, ADInstruments). The contractile response was recorded as the greatest grams of contractile response within the 9-min incubation period. All maximal values measured were corrected for the baseline measured during the interval preceding addition of the 1×10^{-4} *M* norepinephrine reference treatment, thus generating a cumulative concentration-response. To compensate for variation of tissue responsiveness due to differences in tissue size or individual animal variation, values were normalized as a percentage of the maximal contraction produced by norepinephrine. The data are presented as percentage means ± SE of the maximal contraction induced by norepinephrine and plotted to illustrate the response of the bovine lateral saphenous vein.

Data were analyzed using the mixed model procedure (SAS Inst. Inc., Cary, NC) as a completely randomized design. The model included alkaloid, concentration, and the alkaloid × concentration interaction. Comparisons of lysergic acid concentration-response to lysergic acid plus 1×10^{-5} *M* N-acetylloline and 1×10^{-7} *M* ergovaline data were analyzed separately from the corresponding comparison of the ergovaline concentration-response to those containing 1×10^{-5} *M* N-acetylloline and 1×10^{-5} *M* lysergic acid. Statistical comparisons of the N-acetylloline concentration-response to the corresponding lysergic acid and ergovaline combinations were precluded due to differences in the tissue source and procurement. Analysis of variance was conducted, and pairwise comparisons of least squares means (±SEM) were performed if the probability of a greater *F*-statistic was significant for a tested effect. Mean separation was done using the LSD features of SAS. Probabilities of $P < 0.05$ are discussed as significant, unless otherwise noted.

RESULTS AND DISCUSSION

Increasing Concentrations of N-Acetylloline

Previous studies using the methodology and equipment of the current study have been conducted individually for lysergic acid (Klotz et al., 2006) and ergovaline (Klotz et al., 2007), but not for N-acetylloline. Thus, an example of the individual concentration-response for this alkaloid was included along with data obtained using the biopsied venous tissue (Figure 2A). Figure 2B is a magnified section of Figure 2A included to demonstrate the lack of contractile response to increasing concentrations of N-acetylloline (and also gives an enhanced view of the data collection region, beginning immediately after an addition and ending with the first buffer change peak). Data in Figure 3 are presented after normalization to the reference dose of norepinephrine. The N-acetylloline concentration-response (Figure 3) resulted in no detectable contractile response and is similar to previous reports by Abney et al. (1993) using

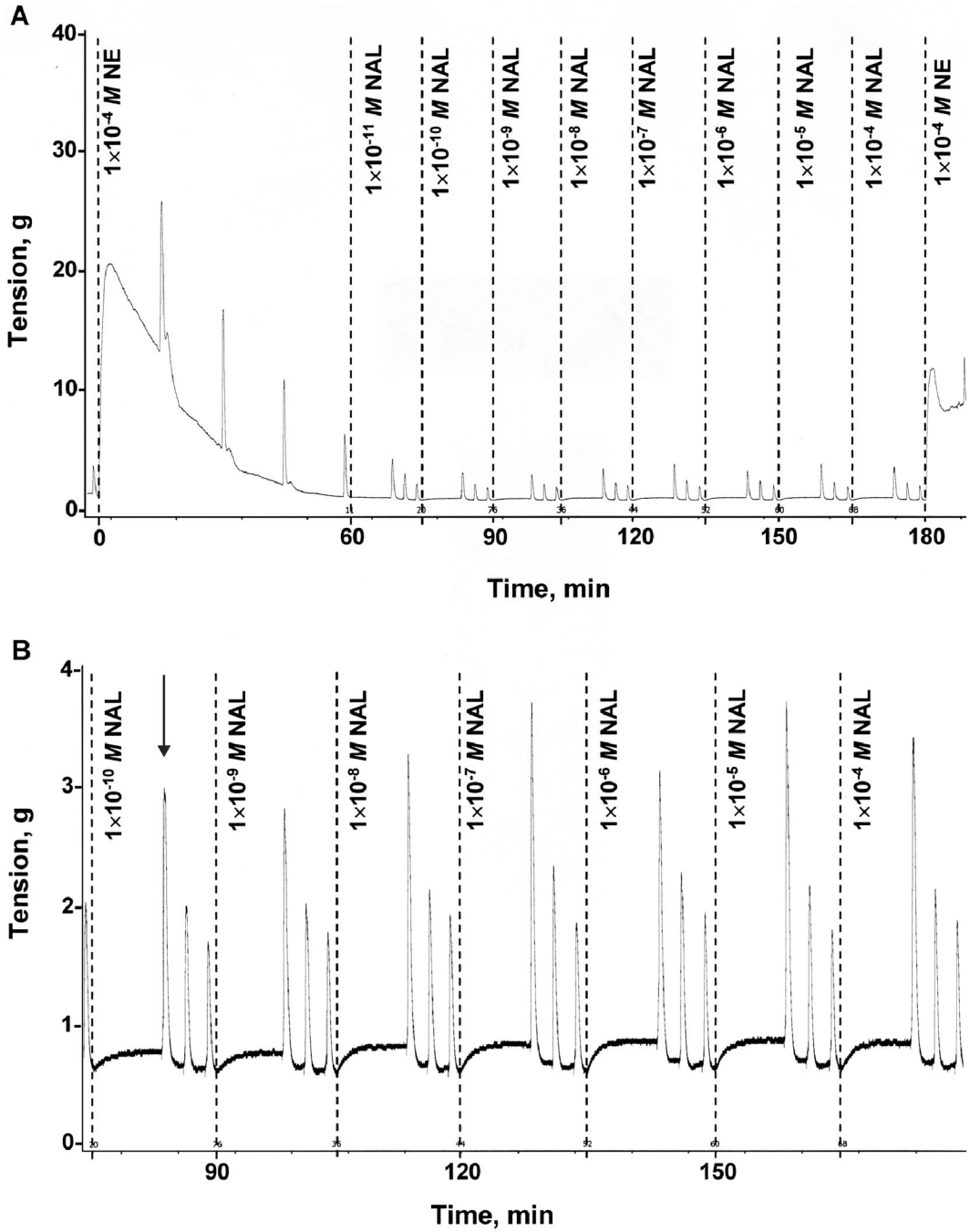

Figure 2. Example of a typical dose response of isolated bovine lateral saphenous vein cross-sections obtained from an abattoir to increasing concentrations of N-acetylloline (NAL, *M*). A) Complete data recording from the myograph that includes the initial addition of norepinephrine (NE), the addition of NAL standards, and the concluding addition of NE. B) A magnified view of 1×10^{-10} to 1×10^{-4} *M* NAL additions. The spikes that precede compound additions (indicated by an arrow) are artifacts generated from buffer replacement and were not included in the data collection and analysis.

equine dorsal metatarsal arteries and Solomons et al. (1989) using bovine dorsal pedal veins. The lack of response in these data justified examining N-acetylloline only in the presence of other alkaloids in biopsied tissues. Also, interest lay primarily with the interaction of N-acetylloline (an unsaturated pyrrolizidine alkaloid) with more reactive ergot alkaloids (e.g., ergovaline), as was suggested by Thompson and Stuedemann (1993) and Porter (1995).

Simultaneous exposure of biopsied bovine lateral saphenous veins to increasing concentrations of N-acetylloline and constant concentrations of lysergic acid vs. constant concentrations of ergovaline generated different responses ($P < 0.01$; Figure 3). The effect of an

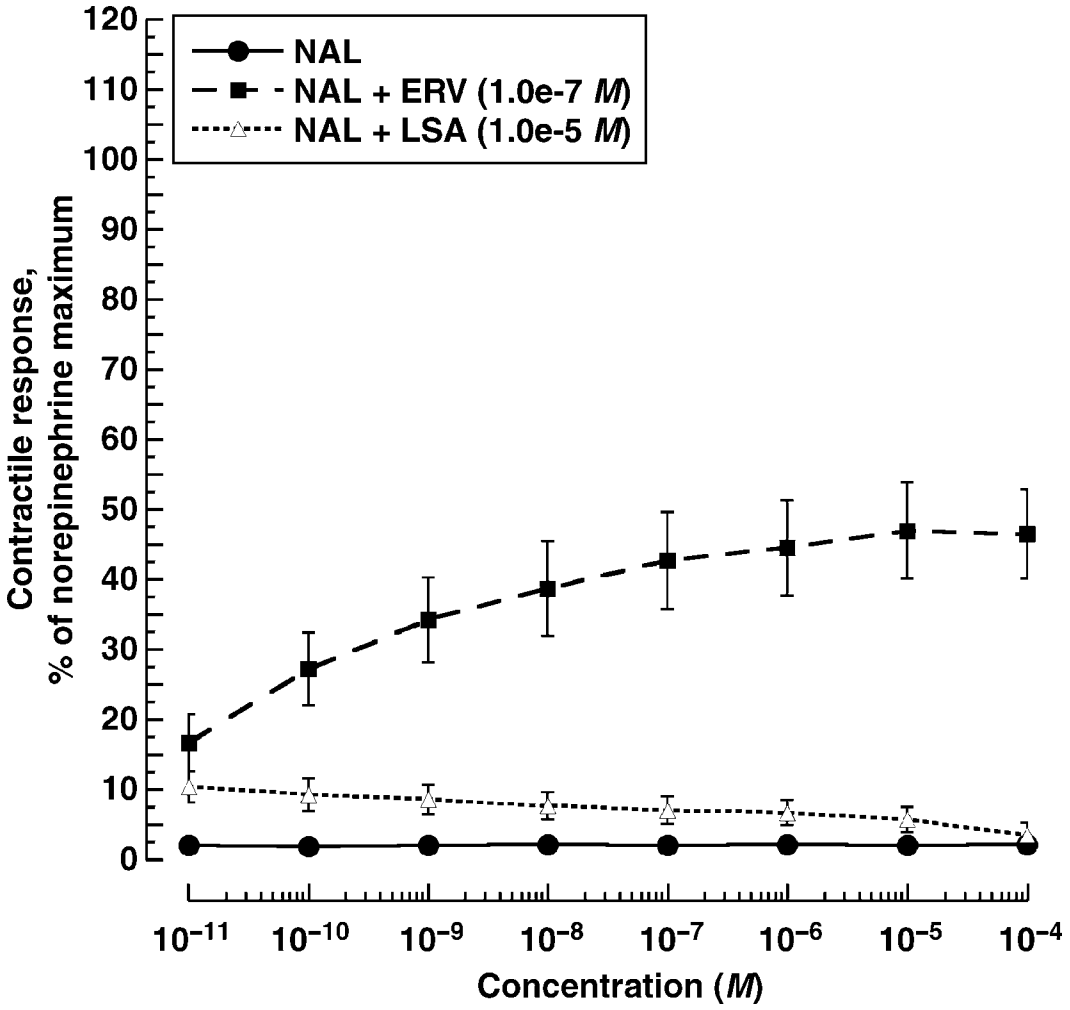

Figure 3. Mean contractile response of bovine lateral saphenous veins to increasing concentrations of N-acetylloline (NAL; ●; n = 4). The tissues used to generate this concentration-response were obtained from abattoir cattle. Increasing concentrations of NAL in combination with 1×10^{-7} *M* ergovaline (ERV; ■; n = 5) and in combination with 1×10^{-5} *M* lysergic acid (LSA; Δ; n = 5). Effects of alkaloid and alkaloid by concentration were significant ($P < 0.01$), and the effect of concentration tended to be significant ($P = 0.09$).

increasing N-acetylloline concentration only tended to differ ($P = 0.09$), with little change within each combination. An alkaloid × concentration interaction was detected ($P = 0.002$), and each paired comparison differed ($P < 0.05$) with the exception of the 2 initial additions of 1×10^{-11} *M* N-acetylloline. This is the obvious result of the 2 combinations resulting in contractile responses that progressed in different directions. The combination of 1×10^{-5} *M* lysergic acid with increasing concentrations of N-acetylloline (Figure 3) resulted in a decreasing contractile response (1×10^{-11} *M* N-acetylloline = 10.2% and 1×10^{-4} *M* N-acetylloline = 3.4% of norepinephrine max) but were not different ($P = 0.30$) from each another. The initial contraction that was observed appeared to approximate the addition of 1×10^{-5} *M* lysergic acid (Figure 4; 6.7% of norepinephrine max) but declined to approximately 3.4%. It is not clear as to why the venous tissue exhibited tachyphylaxsis-like response rather than maintaining a more constant tension that would have been predicted by repetitive additions of 1×10^{-5} *M* lysergic acid alone. Conversely, the combination of 1×10^{-7} *M* ergovaline with increasing concentrations of N-acetylloline resulted in a steadily increasing contraction that reached an asymptote with the final 2 additions (46.8 ± 6.9% of the norepinephrine maximum). The cause of the contractile response in Figure 3 is most likely due to increasing addition of 1×10^{-7} *M* ergovaline and not increasing concentrations of N-acetylloline.

Increasing Concentrations of D-Lysergic Acid

Contractile responses of tall fescue-naïve bovine lateral saphenous veins to increasing concentrations of lysergic acid (Figure 4) generated concentration responses similar to those previously reported using tissues obtained from slaughtered animals (Klotz et al., 2006; maximal contraction of 15.6 ± 2.3% of the norepi-

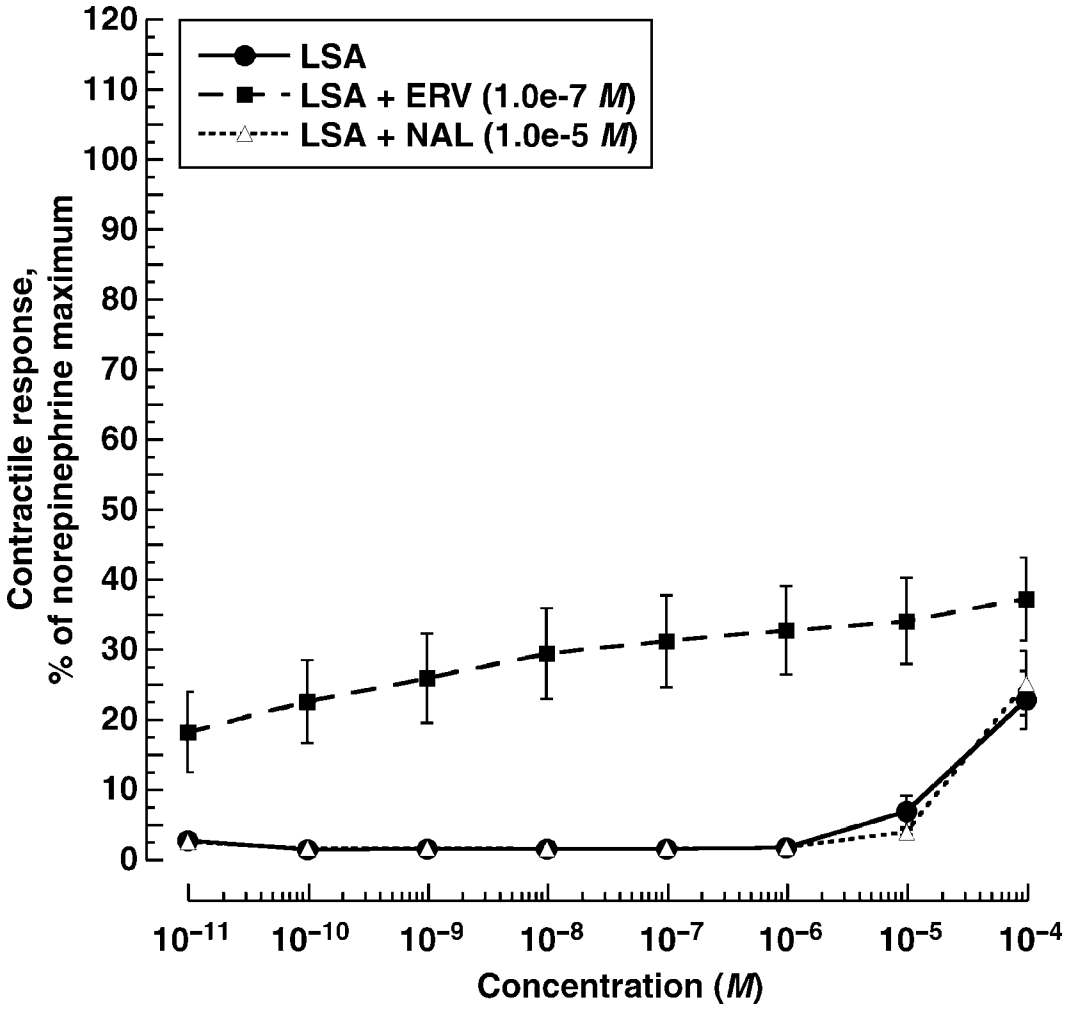

Figure 4. Mean contractile responses of bovine saphenous veins from tall fescue-naïve heifers to increasing concentrations of lysergic acid (LSA; ●; n = 5), increasing concentrations of lysergic acid in combination with 1×10^{-7} *M* ergovaline (ERV; ■; n = 5), and increasing concentrations of lysergic acid in combination with 1×10^{-5} *M* N-acetylloline (NAL; Δ; n = 5). Effects of alkaloid and concentration were significant ($P < 0.01$).

nephrine-induced maximum). Effects of alkaloid and concentration were detected for lysergic acid ($P < 0.01$) but not for alkaloid by concentration ($P = 0.40$). When increasing concentrations of lysergic acid were combined with 1×10^{-5} *M* N-acetylloline (Figure 4; maximal contraction was 24.9 ± 3.8% of the norepinephrine-induced maximum), there was no difference observed from the addition of lysergic acid alone (22.6 ± 3.8% of the norepinephrine-induced maximum). The significant alkaloid effect was likely due to the simultaneous exposure of increasing concentrations of lysergic acid and 1×10^{-7} *M* ergovaline (Figure 4; maximal contraction of 36.9 ± 5.9% of the norepinephrine maximum) being greater than lysergic acid alone or in combination with 1 ± 10^{-5} *M* N-acetylloline ($P < 0.05$). This resembles the response seen with increasing concentrations of N-acetylloline and 1×10^{-7} *M* ergovaline (Figure 3). These responses most likely reflect the cumulative effects of ergovaline exposure. Specifically, the steady increase in tension with each addition and the inability of the segments to return to baseline tension in between additions suggests that a build-up or bioaccumulation of ergovaline could be occurring. This hypothesis is strengthened by previous reports that dissociation of ergovaline from the receptor is very slow (Schöning et al., 2001; Klotz et al., 2007), which could result in increased receptor-binding of ergovaline after repeated exposures.

Moubarak et al. (2003) evaluated effects of simultaneous exposure of ergonovine and ergovaline on the inhibition of Na^+/K^+ ATPase and Mg^{2+} ATPase of the rat kidney and reported an antagonistic interaction between the 2 alkaloids when added simultaneously for inhibition of Na^+/K^+ ATPase but not the Mg^{2+} ATPase. Lysergic acid and ergonovine are both ergoline alkaloids (lysergic acid amides) and are structurally similar. Like the measure of contractile response, evaluation of the inhibition of these enzymes is a method for studying the potency of ergot alkaloids. Unlike the percentage of contractile response seen with lysergic acid (less reac-

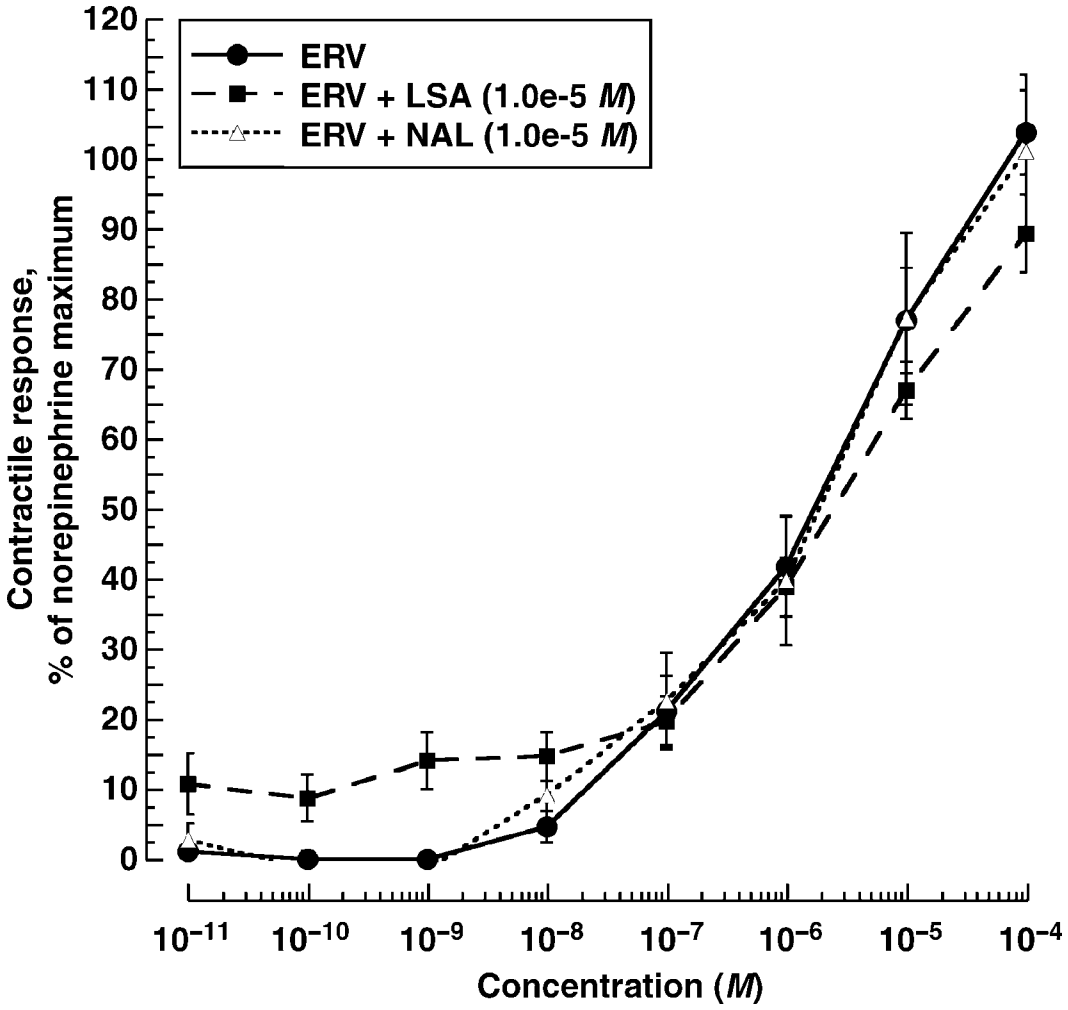

Figure 5. Mean contractile responses of bovine saphenous veins from tall fescue-naïve heifers to increasing concentrations of ergovaline (ERV; ●; n = 5), increasing concentrations of ergovaline in combination with 1×10^{-5} *M* lysergic acid (LSA; ■; n = 5), and increasing concentrations of ergovaline in combination with 1×10^{-5} *M* N-acetylloline (NAL; Δ; n = 5). Effect of concentration was significant ($P < 0.01$).

tive) and ergovaline (more reactive) in the bovine lateral saphenous vein bioassay, the percentage of inhibition in the enzyme activity model (Moubarak et al., 2003) appeared to be more influenced or inhibited by the amount of ergonovine than ergovaline. Although both models demonstrated an interaction between alkaloids in combination, there are still different responses generated by structurally similar alkaloids.

Increasing Concentrations of Ergovaline

Maximal contractile response for ergovaline in the current study was 104.2% (at 1×10^{-4} *M*; Figure 5), and the onset of a contractile response or potency occurred at 1×10^{-8} *M*. Alkaloid and alkaloid × concentration effects were not detected ($P = 0.36$), but effect of concentration was for increasing concentrations of ergovaline ($P < 0.01$). Additions of 1×10^{-5} *M* N-acetylloline and lysergic acid did not affect the contractile response to ergovaline (maximal contractions: 101.5 ± 5.6% and 89.7 ± 5.6% of norepinephrine, respectively). In contrast, addition of 1×10^{-5} *M* lysergic acid caused the response curve to shift up at the 1×10^{-11} *M* concentration of ergovaline. This may be explained by the approximately 6.7 ± 3.8% contractile response to 1×10^{-5} *M* lysergic acid when added alone (Figure 4) plus potentially a small additive effect of ergovaline. Other than this small shift, the concentration-responses of the 2 combinations were nearly identical to that of ergovaline alone, and the response curves were similar to those seen using tissue obtained from abattoir animals (Klotz et al., 2007).

The tetracyclic structure found in both lysergic acid and ergovaline (Figure 1) suggest the potential for similar receptor-binding and the possibility for similar vascular responses. However, this was not observed, and there were no measurable interactions between the 2 when incubated together (Figures 4 and 5). The disparity between the vascular response (i.e., in both the concentration at onset of contraction and the maximal contractile intensity induced) generated by ergovaline and lysergic acid could support the hypothesis that lysergic

acid may be a product of ergovaline degradation. Specifically, the negligible vascular reactivity of lysergic acid compared with ergovaline coupled with reports that greater quantities of lysergic acid are excreted than consumed in both ruminant (De Lorme et al., 2007) and nonruminant (Schultz et al., 2006) herbivores that consumed endophyte-infected tall fescue diets.

Conclusion

The ergovaline and lysergic acid potencies (level at which contraction is initially detected) of 1×10^{-8} and 1×10^{-5} *M*, respectively, resembled those seen using tissue obtained from slaughtered animals (Klotz et al., 2006, 2007). The contractile intensities appeared slightly greater from the fescue-naïve heifers than the abattoir cattle (22.6 ± 4.1% and 104.2 ± 6.0% vs. 15.6 ± 2.3% and 69.6 ± 5.3% of the norepinephrine maximum for lysergic acid and ergovaline, respectively), and this warrants further investigation. There did appear to be some interaction of the alkaloids when the tissue was exposed to various combinations, because combinations containing 1×10^{-7} *M* ergovaline differed from individual concentration responses. Conversely, it appears that N-acetylloline did not inhibit or potentiate the effects of ergot alkaloids on vascular activity to any appreciable extent. These findings support the possibility that at least an additive effect may exist, and this should be considered when using in vitro bioassays and interpreting resultant data. Further research is needed to investigate the possibility that bioaccumulation of ergovaline could be occurring in this bioassay and in animals consuming endophyte-infected tall fescue.

LITERATURE CITED

Abney, L. K., J. W. Oliver, and C. R. Reinemeyer. 1993. Vasoconstrictive effects of tall fescue alkaloids on equine vasculature. J. Equine Vet. Sci. 13:334–340.

De Lorme, M. J. M., S. L. Lodge-Ivey, and A. M. Craig. 2007. Physiological and digestive effects of *Neotyphodium coenophialum*-infected tall fescue fed to lambs. J. Anim. Sci. 85:1199–1206.

Dyer, D. C. 1993. Evidence that ergovaline acts on serotonin receptors. Life Sci. 53:223–228.

Klotz, J. L., L. P. Bush, D. L. Smith, W. D. Shafer, L. L. Smith, B. C. Arrington, and J. R. Strickland. 2007. Ergovaline-induced vasoconstriction in an isolated bovine lateral saphenous vein bioassay. J. Anim. Sci. 85:2330–2336.

Klotz, J. L., L. P. Bush, D. L. Smith, W. D. Shafer, L. L. Smith, A. C. Vevoda, A. M. Craig, B. C. Arrington, and J. R. Strickland. 2006. Assessment of vasoconstrictive potential of D-lysergic acid using an isolated bovine lateral saphenous vein bioassay. J. Anim. Sci. 84:3167–3175.

Moubarak, A. S., Z. B. Johnson, and C. F. Rosenkrans Jr. 2003. Antagonistic effects of simultaneous exposure of ergot alkaloids on kidney adenosine triphosphatase system. In Vitro Cell. Dev. Biol. Anim. 39:395–398.

Oliver, J. W. 1997. Physiological manifestations of endophyte toxicosis in ruminant and laboratory species. Pages 311–346 in C. W. Bacon and N. S. Hill, ed. *Neotyphodium*/Grass Interactions. Plenum Publ., New York, NY.

Oliver, J. W., L. K. Abney, J. R. Strickland, and R. D. Linnabary. 1993. Vasoconstriction in bovine vasculature induced by the tall fescue alkaloid lysergamide. J. Anim. Sci. 71:2708–2713.

Oliver, J. W., J. R. Strickland, J. C. Waller, H. A. Fribourg, R. D. Linnabary, and L. K. Abney. 1998. Endophytic fungal toxin effect on adrenergic receptors in lateral saphenous veins (cranial branch) of cattle grazing tall fescue. J. Anim. Sci. 76:2853–2856.

Porter, J. K. 1995. Analysis of endophyte toxins: Fescue and other grasses toxic to livestock. J. Anim. Sci. 73:871–880.

Schöning, C., M. Flieger, and H. H. Pertz. 2001. Complex interaction of ergovaline with 5-HT2A, 5-HT1B/1D, and α1 receptors in isolated arteries of rat and guinea pig. J. Anim. Sci. 79:2202–2209.

Schultz, C. L., S. L. Lodge-Ivey, L. P. Bush, A. M. Craig, and J. R. Strickland. 2006. Effects of initial and extended exposure to an endophyte-infected tall fescue seed diet on faecal and urinary excretion of ergovaline and lysergic acid in mature geldings. N. Z. Vet. J. 54:178–184.

Solomons, R. N., J. W. Oliver, and R. D. Linnabary. 1989. Reactivity of the dorsal pedal vein of cattle to selected alkaloids associated with *Acremonium coenophialum*-infected fescue grass. Am. J. Vet. Res. 50:235–238.

Soreng, R. J., E. E. Terrell, J. Wiersema, and S. J. Darbyshire. 2001. Proposal to conserve the name *Schedonorus arundinaceus* (Schreb.) Dumort. against *Schedonorus arundinaceus* Roem. & Schult. (*Poaceae: Poeae*). Taxon 50:915–917.

Thompson, F. N., and J. A. Stuedemann. 1993. Pathophysiology of fescue toxicosis. Agric. Ecosyst. Environ. 44:263–281.